SJ-4

CORRECTED VERSION*

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/55, 15/63, 1/21, 5/10, 9/18, A61K 48/00, C12Q 1/68, 1/44

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US 60/075,258 (CIP)
Filed on 19 February 1998 (19.02.98)

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(72) Inventors; and
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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
With amended claims.

(54) Title: COMPOSITIONS AND METHODS FOR SENSITIZING AND INHIBITING GROWTH OF HUMAN TUMOR CELLS

(57) Abstract

Polynucleotides encoding a carboxylesterase enzyme and polypeptides encoded by the polynucleotides which are capable of metabolizing a chemotherapeutic prodrug and inactive metabolites thereof to active drug are provided. Compositions and methods for sensitizing tumor cells to a prodrug chemotherapeutic agent and inhibiting tumor growth with this enzyme are also provided. In addition, screening assay for identification of drugs activated by this enzyme are described.

PATENT COOPERATION TREATY

RECEIVED
FEB 07 2000

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To: JANE MASSEY LICATA
LAW OFFICES OF JANE MASSEY LICATA
66 E. MAIN STREET
MARLTON, NEW JERSEY 08053

Docket System ✓
Status Report ✓
Docket Book ✓
4/3/00

WRITTEN OPINION

(PCT Rule 66)

Date of Mailing *(day/month/year)*	03 FEB 2000
Applicant's or agent's file reference: SJ-0004	REPLY DUE within TWO months from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/03171	12 FEBRUARY 1999	19 FEBRUARY 1998

International Patent Classification (IPC) or both national classification and IPC
Please See Supplemental Sheet.

Applicant
ST. JUDE CHILDREN'S RESEARCH HOSPITAL

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I [X] Basis of the opinion
- II [ ] Priority
- III [X] Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
- IV [X] Lack of unity of invention
- V [X] Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [ ] Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiratio of that time limit, request this Authority to grant an extension., see Rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 19 JUNE 2000

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	REBECCA BROUTY
Facsimile N . (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/408 (cover sheet) (January 1994)*

WRITTEN OPINION

International application No.
PCT/US99/03171

I. Basis of the opinion

1. This opinion has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".)*:

☐ the international application as originally filed.

☒ the description, pages (See Attached) , as originally filed.
pages _____ , filed with the demand.
pages _____ , filed with the letter of _____.

☒ the claims, Nos. (See Attached) , as originally filed.
Nos. _____ , as amended under Article 19.
Nos. _____ , filed with the demand.
Nos. _____ , filed with the letter of _____.

☒ the drawings, sheets~~/fig~~ (See Attached) , as originally filed.
sheets~~/fig~~ _____ , filed with the demand.
sheets~~/fig~~ _____ , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets~~/fig~~ NONE

3. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:
NONE

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 20-22

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _ are so unclear that no meaningful opinion could be formed *(specify)*.

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ an international search report has been established for said claims Nos. 20-22.

WRITTEN OPINION

International application No.
PCT/US99/03171

IV. Lack f unity of invention

1. In response to the invitation (F rm PCT/IPEA/405) to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.

2. This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1 not to invite the applicant to restrict or pay additional fees:

Please See Supplemental Sheet.

3. C nsequently, the following parts f the international appiication were the subject of international preliminary examinati n in establishing this pinion:

- ☒ all parts.
- ☐ the parts relating to claims Nos. _.

WRITTEN OPINION

International application No.
PCT/US99/03171

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; itations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims	8-17	YES
	Claims	1-7, 18, 19	NO
Inventive Step (IS)	Claims	none	YES
	Claims	1-19	NO
Industrial Applicability (IA)	Claims	1-19	YES
	Claims	none	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-7, 18 and 19 lack novelty under PCT Article 33(2) as being anticipated by Danks et al.

Danks et al. teach that a rabbit liver carboxylesterase (which by footnote 4 is identical to that of Figure 4) as well as the commercially available Sigma Chemical Co. rabbit liver carboxylesterase are capable of metabolizing the prodrug CPT-11 to its active SN-38 form *in vitro* and that human tumor cells transfected with a cDNA encoding the rabbit liver carboxylesterase of Figure 4 are more sensitive to CPT-11 than untransfected cells. They further teach assays for screening for hydrolysis of the ester bond of CPT-11 as claimed in Claim 19.

Claims 7 and 19 lack novelty under PCT Article 33(2) as being anticipated by Senter et al.

Senter et al. teach that a rat serum carboxylesterase identical to the protein of Alekson et al. is capable of metabolizing the prodrugs paclitaxel-2'-ethylcarbonate and CPT-11 to their active paclitaxel and SN-38 forms *in vitro* and human and mouse tumor cells are more sensitive to the prodrugs in the presence of this enzyme than in its absence. They further teach assays for screening for hydrolysis of the ester bond of paclitaxel-2'-ethylcarbonate as claimed in Claim 19.

Claims 1, 4-7, 18 and 19 lack novelty under PCT Article 33(2) as being anticipated by Alekson et al.

Alekson et al. teach a rat serum carboxylesterase and cDNA, vectors and host cells encoding this enzyme. This carboxylesterase is capable of metabolizing the prodrugs paclitaxel-2'-ethylcarbonate and CPT-11 to their active paclitaxel and SN-38 forms *in vitro* (see Senter et al.) and thus meets all limitations of the instant claims.

Claim 7 lacks novelty under PCT Article 33(2) as being anticipated by Miller et al. or Sigma Chemical Co. Catalog Products E2884 and E9636.

(Continued on Supplemental Sheet.)

WRITTEN OPINION

International application No.
PCT/US99/03171

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

TIME LIMIT:

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12N 15/55, 15/63, 1/21, 5/10, 9/18; A61K 48/00; C12Q 1/68, 1/44 and US Cl.: 536/23.2; 435/320.1, 252.3, 325, 197, 6, 19; 514/44

I. BASIS OF OPINION:

This opinion has been drawn on the basis of the description,
pages, 1-34, as originally filed.
pages, none, filed with the demand.
and additional amendments:
none

This opinion has been drawn on the basis of the claims,
numbers, 1-18 , as originally filed.
numbers, NONE, as amended under Article 19.
numbers, 19-22, filed with the demand.
and additional amendments:
none

This opinion has been drawn on the basis of the drawings,
sheets, 1-12, as originally filed.
sheets, none, filed with the demand.
and additional amendments:
none

IV. LACK OF UNITY OF INVENTION:

2. Although this IPEA did not invite applicant to restrict or pay additional fees, Unity of Invention is lacking for the following reasons:
This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-6, and 8-11, drawn to DNA, vectors and host cells encoding a carboxylesterase which is capable of metabolizing a chemotherapuetic prodrug to the active drug and methods of use thereof for sensitizing tumor cells to a chemotherapuetic prodrug.
Group II, claim 7, drawn to a carboxylesterase which is capable of metabolizing a chemotherapuetic prodrug to the active drug.
Group III, claims 12, 13, and 17, drawn to methods of inhibiting tumor growth.
Group IV, claims 14 and 15, drawn to methods of inhibiting tumor recurrence.
Group V, claim 16, drawn to methods of purging bone marrow cells.
Group VI, claims 18 and 19, drawn to methods of assaying for drugs activated by carboxylesterase.

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The DNA of Group I and protein of Group II do not share a corresponding special technical feature even though the DNA encodes the protein because the prior art clearly teaches a carboxylesterase which is capable of metabolizing a chemotherapuetic prodrug to the active drug (see for example the 1994 Sigma catalog, entries E2884 and E9686). Therefore the shared technical feature of these claims, i.e., the carboxylesterase, does not constitute a special technical feature as defined in PCT Rule 13.2 as it is not a feature

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuati n of: Boxes I - VIII

which defines a contribution the claimed inventions make over the prior art. The methods of Groups III-VI do not share any technical feature with Group II and do not have unity of invention with Group I as Group I already includes a method of use of the carboxylesterase DNA which comprises unrelated steps to the methods of Groups III-VI and 37 CFR 1.475 does not provide for the inclus . n of multiple methods of use within the main invention.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

Miller et al. and Sigma Chemical Co. Catalog Products E2884 and E9636 disclose a rabbit liver carboxylesterase. This carboxylesterase is capable of metabolizing the prodrug CPT-11 to its active SN-38 form *in vitro* (see Danks et al.) and thus meets all limitations of the instant claim.

Claims 8-17 lack an inventive step under PCT Article 33(3) as being obvious over Danks et. al. or Senter et al. and Alekson et al. in view of Mullen et al. (WO 93/01281).

Danks et. al., Senter et al. and Alekson et al. are all discussed above. Danks et. al. and Senter et al. each suggest that the carboxylesterase enzymes and genes encoding therefore could be used for *in vivo* targeting of tumor cells for killing by the active drug by targeting the enzyme to the tumor cells or enhancing the activity of the enzyme in the tumor cells followed by administration of the prodrug. Danks et al. specifically suggest that the genes encoding the activating carboxylesterase could be used in similar fashion to the approach used in other art prodrug/activating enzyme combinations, such as ganciclovir/HSV thymidine kinase and 5-fluorocytosine/cytosine deaminase.

Mullen et al. teach methods and compositions therefor for the treatment of tumors or for purging bone marrow of tumor cells with prodrug/activating enzyme combinations using 5-fluorocytosine/cytosine deaminase comprising administration to a patient or to bone marrow *in vitro* of a DNA construct comprising a tumor specific promoter and a gene encoding the enzyme and subsequent treatment with the prodrug.

Therefore, in view of the explicit suggestion by Danks et al. and Senter et al. therefore, it would have been obvious to one of ordinary skill in the art to substitute a gene encoding the carboxylesterases which activate CPT-11 for the cytosine deaminase genes of Mullen et al. and to substitute CPT-11 for 5-fluorocytosine in order to develop a tumor treatment strategy for CPT-11.

---------- NEW CITATIONS ----------

NONE

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference SJ-0004	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/03171	International filing date *(day/month/year)* 12 FEBRUARY 1999	Priority date *(day/month/year)* 19 FEBRUARY 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant ST. JUDE CHILDREN'S RESEARCH HOSPITAL		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☒ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☒ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14 SEPTEMBER 1999	23 MAY 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer REBECCA PROUTY Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/03171

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages (See Attached) , as originally filed
pages _____ , as amended (together with any statement) under Article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the drawings:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the sequence listing part of the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 20-22

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _ are so unclear that no meaningful opinion could be formed *(specify)*.

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 20-22 .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- [ ] restricted the claims.
- [ ] paid additional fees.
- [ ] paid additional fees under protest.
- [ ] neither restricted nor paid additional fees.

2. [X] This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- [ ] complied with.
- [X] not complied with for the following reasons:

Please See Supplemental Sheet.

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- [X] all parts.
- [ ] the parts relating to claims Nos. _.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	8-17	YES
	Claims	1-7, 18, 19	NO
Inventive Step (IS)	Claims	none	YES
	Claims	1-19	NO
Industrial Applicability (IA)	Claims	1-19	YES
	Claims	none	NO

2. citations and explanations (Rule 70.7)

Claims 1-7, 18 and 19 lack novelty under PCT Article 33(2) as being anticipated by Danks et al.

Danks et al. teach that a rabbit liver carboxylesterase (which by footnote 4 is identical to that of Figure 4) as well as the commercially available Sigma Chemical Co. rabbit liver carboxylesterase are capable of metabolizing the prodrug CPT-11 to its active SN-38 form *in vitro* and that human tumor cells transfected with a cDNA encoding the rabbit liver carboxylesterase of Figure 4 are more sensitive to CPT-11 than untransfected cells. They further teach assays for screening for hydrolysis of the ester bond of CPT-11 as claimed in Claim 19.

Claims 7 and 19 lack novelty under PCT Article 33(2) as being anticipated by Senter et al.

Senter et al. teach that a rat serum carboxylesterase identical to the protein of Alekson et al. is capable of metabolizing the prodrugs paclitaxel-2'-ethylcarbonate and CPT-11 to their active paclitaxel and SN-38 forms *in vitro* and human and mouse tumor cells are more sensitive to the prodrugs in the presence of this enzyme than in its absence. They further teach assays for screening for hydrolysis of the ester bond of paclitaxel-2'-ethylcarbonate as claimed in Claim 19.

Claims 1, 4-7, 18 and 19 lack novelty under PCT Article 33(2) as being anticipated by Alekson et al.

Alekson et al. teach a rat serum carboxylesterase and cDNA, vectors and host cells encoding this enzyme. This carboxylesterase is capable of metabolizing the prodrugs paclitaxel-2'-ethylcarbonate and CPT-11 to their active paclitaxel and SN-38 forms *in vitro* (see Senter et al.) and thus meets all limitations of the instant claims.

Claim 7 lacks novelty under PCT Article 33(2) as being anticipated by Miller et al. or Sigma Chemical Co. Catalog Products E2884 and E9636.

(Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12N 15/55, 15/63, 1/21, 5/10, 9/18; A61K 48/00; C12Q 1/68, 1/44 and US Cl.: 536/23.2; 435/320.1, 252.3, 325, 197, 6, 19; 514/44

I. BASIS OF REPORT:

This report has been drawn on the basis of the description,
page(s) 1-34, as originally filed.
page(s) none, filed with the demand.
and additional amendments:
none

This report has been drawn on the basis of the claims,
page(s) 35-36, as originally filed.
page(s) 37, as amended under Article 19.
page(s) none, filed with the demand.
and additional amendments:
none

This report has been drawn on the basis of the drawings,
page(s) 1-12, as originally filed.
page(s) none, filed with the demand.
and additional amendments:
none

This report has been drawn on the basis of the sequence listing part of the description:
page(s) NONE, as originally filed.
pages(s) NONE, filed with the demand.
and additional amendments:
NONE

IV. LACK OF UNITY OF INVENTION:

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2, and 13.3 is not complied with for the following reasons:
This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-6, and 8-11, drawn to DNA, vectors and host cells encoding a carboxylesterase which is capable of metabolizing a chemotherapuetic prodrug to the active drug and methods of use thereof for sensitizing tumor cells to a chemotherapuetic prodrug.
Group II, claim 7, drawn to a carboxylesterase which is capable of metabolizing a chemotherapuetic prodrug to the active drug.
Group III, claims 12, 13, and 17, drawn to methods of inhibiting tumor growth.
Group IV, claims 14 and 15, drawn to methods of inhibiting tumor recurrence.
Group V, claim 16, drawn to methods of purging bone marrow cells.
Group VI, claims 18 and 19, drawn to methods of assaying for drugs activated by carboxylesterase.

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The DNA of Group I and protein of Group II do not share a corresponding special technical feature even though the DNA encodes the protein because the prior art clearly teaches a carboxylesterase which is capable of metabolizing a chemotherapuetic prodrug to the active drug (see for example the 1994 Sigma catalog, entries E2884 and E9686). Therefore the shared technical feature of these claims, i.e., the carboxylesterase, does not constitute a special technical feature as defined in PCT Rule 13.2 as it is not a feature

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

which defines a contribution the claimed inventions make over the prior art. The methods of Groups III-VI do not share any technical feature with Group II and do not have unity of invention with Group I as Group I already includes a method of use of the carboxylesterase DNA which comprises unrelated steps to the methods of Groups III-VI and 37 CFR 1.475 does not provide for the inclusion of multiple methods of use within the main invention.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

Miller et al. and Sigma Chemical Co. Catalog Products E2884 and E9636 disclose a rabbit liver carboxylesterase. This carboxylesterase is capable of metabolizing the prodrug CPT-11 to its active SN-38 form *in vitro* (see Danks et al.) and thus meets all limitations of the instant claim.

Claims 8-17 lack an inventive step under PCT Article 33(3) as being obvious over Danks et. al. or Senter et al. and Alekson et al. in view of Mullen et al. (WO 93/01281).

Danks et. al., Senter et al. and Alekson et al. are all discussed above. Danks et. al. and Senter et al. each suggest that the carboxylesterase enzymes and genes encoding therefore could be used for *in vivo* targeting of tumor cells for killing by the active drug by targeting the enzyme to the tumor cells or enhancing the activity of the enzyme in the tumor cells followed by administration of the prodrug. Danks et al. specifically suggest that the genes encoding the activating carboxylesterase could be used in similar fashion to the approach used in other art prodrug/activating enzyme combinations, such as ganciclovir/HSV thymidine kinase and 5-fluorocytosine/cytosine deaminase.

Mullen et al. teach methods and compositions therefor for the treatment of tumors or for purging bone marrow of tumor cells with prodrug/activating enzyme combinations using 5-fluorocytosine/cytosine deaminase comprising administration to a patient or to bone marrow *in vitro* of a DNA construct comprising a tumor specific promoter and a gene encoding the enzyme and subsequent treatment with the prodrug.

Therefore, in view of the explicit suggestion by Danks et al. and Senter et al. therefore, it would have been obvious to one of ordinary skill in the art to substitute a gene encoding the carboxylesterases which activate CPT-11 for the cytosine deaminase genes of Mullen et al. and to substitute CPT-11 for 5-fluorocytosine in order to develop a tumor treatment strategy for CPT-11.

---------- NEW CITATIONS ----------

NONE

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/03171

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	MILLER, S.K. et al. Purification and Physical Properties of Oligomeric and Monomeric Carboxylesterases from Rabbit Liver. J. Biol. Chem. 10 August 1980, Vol. 255, No. 15, pages 7161-7167, see entire document.	7 --- 1-6, 8-19
X,P --- Y,P	POTTER, P.M. et al. Isolation and Partial Characterization of a cDNA Encoding a Rabbit Liver Carboxylesterase That Activates the Prodrug Irinotecan (CPT-11). Cancer Research 15 June 1998, Vol. 58, pages 2646-2651, see entire document.	1, 2, 4-7 --------- 3, 8-19
X,P ---- Y,P	POTTER, P.M. et al. Isolation and Characterization of a cDNA Encoding a Rabbit Carboxylesterase That Converts CPT-11 to SN-38. Proc. Amer. Assoc. Canc. Res. March 1998, Vol 39, page 421, see entire document.	1, 2, 4-7 ---------- 3, 8-19
X - Y	DANKS, M.K. et al. Overexpression of a Rabbit Liver Carboxylesterase Sensitizes Human Tumor Cells to CPT-11 Cancer Research 01 January 1998, Vol. 58, pages 20-22, see entire document.	1, 2, 4-7 ---------- 3, 8-19
X,P --- Y,P	POTTER, P.M. et al. Cellular Localization Domains of a Rabbit and a Human Carboxylesterase: Influence on Irinotecan (CPT-11) Metabolism by the Rabbit Enzyme. Cancer Research. 15 August 1998, Vol. 58, pages 3627-3632, see entire document.	1-7 ---- 8-19
Y	WO 93/01281 A1 (MULLEN et al.) 21 January 1993, especially paes 4-6, 17, 21 and 22.	8-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/03171

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 26 October 1999 (26.10.99)	
International application No. PCT/US99/03171	**Applicant's or agent's file reference** SJ-0004
International filing date (day/month/year) 12 February 1999 (12.02.99)	**Priority date** (day/month/year) 19 February 1998 (19.02.98)
Applicant DANKS, Mary, K. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

14 September 1999 (14.09.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Forax
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

09/622568

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

U.S. APPLICATION NO.		FIRST NAMED APPLICANT	ATTY. DOCKET NO.
09/622,568		SHINKS	

INTERNATIONAL APPLICATION NO.
PCT/US99/01017

I.A. FILING DATE	PRIORITY DATE
02/12/99	02/12/98

5071

JANE MASSEY LICATA
66 E MAIN STREET
MARLTON NJ 08053

DATE MAILED: 11/15/00

NOTIFICATION OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

1. The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as ☐ a Designated Office (37 CFR 1.494), ☑ an Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is ACCEPTED for national patentability examination in the United States Patent and Trademark Office.

2. The United States Application Number assigned to the application is shown above and the relevant dates are:

31 Aug 00
35 U.S.C. 102(e) DATE

31 Aug 00
DATE OF RECEIPT OF 35 U.S.C. 371 REQUIREMENTS

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE "FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371(C) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** *The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363).* Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

3. ☐ A request for immediate examination under 35 U.S.C. 371(f) was received on _____ and the application will be examined in turn.

4. The following items have been received:
- ☑ U.S. Basic National Fee.
- ☑ Copy of the international application in:
  - ☐ a non-English language.
  - ☑ English.
- ☐ Translation of the international application into English.
- ☑ Oath or Declaration of inventors(s) for DO/EO/US.
- ☑ Copy of Article 19 amendments. ☐ Translation of Article 19 amendments into English.
  The Article 19 amendments ☐ have ☐ have not been entered.
- ☐ The International Preliminary Examination Report in English and its Annexes, if any.
- ☐ Copy of the Annexes to the International Preliminary Examination Report (IPER).
  - ☐ Translation of Annexes to the IPER into English.
  The Annexes ☐ have ☐ have not been entered.
- ☐ Preliminary amendment(s) filed _____ and _____.
- ☑ Information Disclosure Statement(s) filed 15 Aug 00 and _____.
- ☐ Assignment document.
- ☐ Power of Attorney and/or Change of Address.
- ☐ Substitute specification filed _____.
- ☑ Statement Claiming Small Entity Status.
- ☐ Priority Document.
- ☑ Copy of the International Search Report ☐ and copies of the references cited therein.
- ☑ Other: Sequence Listing

Applicant is reminded that any communication to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above. (37 CFR 1.5)

Maya Baltimore
National Stage Processing
Telephone: (703) 305-3095

FORM PCT/DO/EO/903 (December 1997)

09/622568

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: ASSISTANT COMMISSIONER FOR PATENTS
Box PCT
Washington, D.C. 20231

U.S. APPLICATION NO.		FIRST NAMED APPLICANT	ATTY. DOCKET NO.
09/622,568		DANKS M	SJ 0011

INTERNATIONAL APPLICATION NO.
PCT/US99/03171

I.A. FILING DATE	PRIORITY DATE
02/12/99	02/19/98

5071

JANE MASSEY LICATA
56 E MAIN STREET
MARLTON NJ 08053

DATE MAILED: 10/23/00

NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

1. The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as ☐ a Designated Office (37 CFR 1.494), ☑ an Elected Office (37 CFR 1.495):

- ☑ U.S. Basic National Fee.
- ☑ Copy of the international application in:
  - ☐ a non-English language.
  - ☑ English.
- ☐ Translation of the international application into English.
- ☐ Oath or Declaration of inventors(s) for DO/EO/US.
- ☑ Copy of Article 19 amendments.
- ☐ Translation of Article 19 amendments into English.
- ☐ The International Preliminary Examination Report in English and its Annexes, if any.
- ☐ Translation of Annexes to the International Preliminary Examination Report into English.
- ☐ Preliminary amendment(s) filed _____ and _____.
- ☑ Information Disclosure Statement(s) filed _____ and _____.
- ☐ Assignment document.
- ☐ Power of Attorney and/or Change of Address.
- ☐ Substitute specification filed _____.
- ☑ Statement Claiming Small Entity Status.
- ☐ Priority Document.
- ☑ Copy of the International Search Report ☐ and copies of the references cited therein.
- ☑ Other: Sequence listing

2. The following items MUST be furnished within the period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

- ☐ a. Translation of the application into English. Note a processing fee will be required if submitted later than the appropriate 20 or 30 months from the priority date.
  - ☐ The current translation is defective for the reasons indicated on the attached Notice of Defective Translation.
- ☐ b. Processing fee for providing the translation of the application and/or the Annexes later that the appropriate 20 or 30 months from the priority date (37 CFR 1.492(f)).
- ☑ c. Oath or declaration of the inventors, in compliance with 37 CFR 1.497(a) and (b), identifying the application by the International application number and international filing date.
  - ☐ The current oath or declaration does not comply with 37 CFR 1.497(a) and (b) for the reasons indicated on the attached PCT/DO/EO/917.
- ☑ d. Surcharge for providing the oath or declaration later that the appropriate 20 or 30 months from the priority date (37 CFR 1.492(e)).

3. Additional claim fees of $_____ as a ☐ large entity ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due (37 CFR 1.492(g)). See attached PTO-875.

ALL OF THE ITEMS SET FORTH IN 2(a)-2(d) AND 3 ABOVE MUST BE SUBMITTED WITHIN ONE MONTH FROM THE DATE OF THIS NOTICE OR BY ☐ 21 OR ☑ 31 MONTHS FROM THE PRIORITY DATE FOR THE APPLICATION, WHICHEVER IS LATER. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

4. Translation of the Annexes MUST be submitted no later that the time period set above or the annexes will be cancelled. Note processing fee will be required if submitted later than 30 months from the priority date.

5. ☐ The Article 19 amendments are cancelled since a translation was not provided by the appropriate 20 (37 CFR 1.494(d)) or 30 (37 CFR 1.495(d)) months from the priority date.

Applicant is reminded that any communication to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above. (37 CFR 1.5)

A copy of this notice MUST be returned with this response.

Enclosed: ☐ PCT/DO/EO/917 ☐ Notice of Defective Translation
☐ PTO-875

FORM PCT/DO/EO/905 (December 1997)

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